ALTERATIONS OF CATALASE ACTIVITY IN PINUS LEAVES

by

DR. V. FRENYÓ and J. MIHÁLYFI

Department of Plant Physiology of the Eötvös Loránd University, Budapest

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Introduction

In his investigations concerning the tissues of *Vitis vinifera* Jerzy Czosnowski (1952) has found remarkable changes, taking place in the activity of enzymes as a result of biochemical and physiological conditions; his findings were published in "Poznánskie Towarzystwo Przyjaciól Nauk" by the Polish Academy of Sciences. In Hungary, catalase activity of *Vitis* has been thoroughly dealt with by Mária Horváth-Mészáros, (1961) who studied the effect of hybridization. Our own results have confirmed the conclusion to be drawn from the work of the Polish and Hungarian authors referred to: enzyme activity can actually be used for diagnostical purposes (Frenyó 1962; Frenyó and Szendrői 1963). This result asserts itself in the present paper summing up a finished part of a more comprehensive test series. Catalase activity can furnish informations, for instance, on metabolic alterations resulting from different treatments of seedlings grown in nurseries (Horváth – Novák, 1962). For this purpose, enzyme activity must be known by the parts of the body and by the sections of the day. With the present paper we wish to contribute to the foundations of a diagnostical procedure to be developed later.

Material and method

The examinations have been performed in the field by means of an appliance patented under the Hungarian licence No. FE–542/42. The essence of the method is to place the tissue pieces to be tested into one of the branches of a Y-shaped small glass recipient and to pour hydrogen peroxide solution into the other branch. A scaled capillary measuring tube is adapted to the polished inlet of the recipient. If the recipient is now overturned, the solution, separated so far on account of the Y-shape, comes into contact with the tissue pieces.

Dependent on catalase activity of the wound surfaces, the following reaction takes place in a shorter or longer time:

$$2\,H_2O_2 \longrightarrow 2\,H_2O + O_2$$

Released O_2 drives as much liquid during a unit time into the measuring tube turned downwards, as corresponds to the volume of the released gas. Thus, the technique is based on the principle of gasometry.

The recipient may have also another shape than that of Y (Fig. 1.)

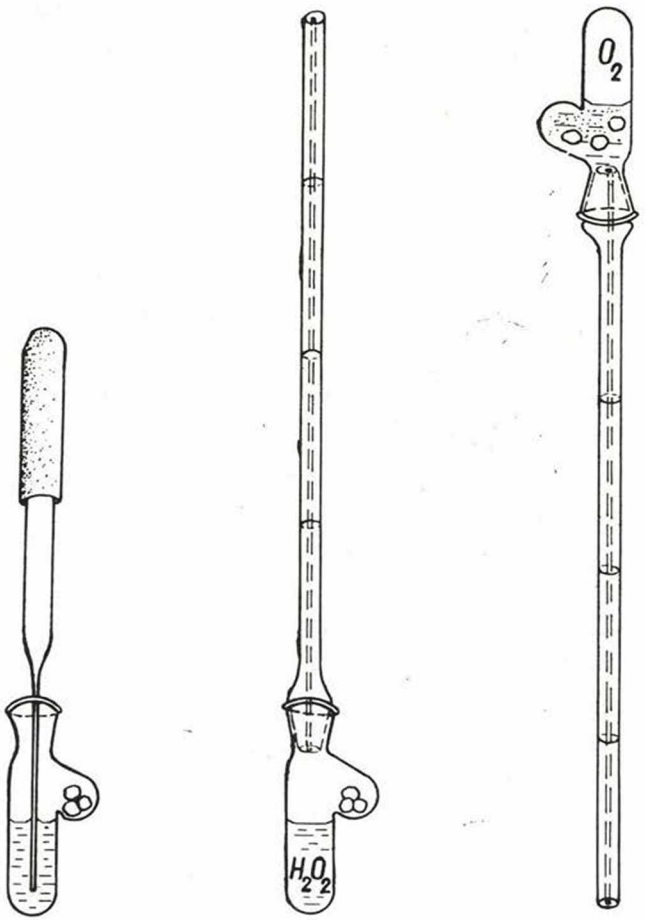

Fig. 1. A type of the catalase-testing device of F r e n y ó. The hollow side is for the test substance (e. g. leaf disks). 1% hydrogen-hyperoxide solution is poured into the straight part. The capillary measuring tube is inserted and the device is used in overturned position to measure the amount of liquid driven by released O_2 during a unit time into the measuring tube turned downwards

The pine-needles involved in the present test have been divided into apical, medial and basal parts, each of which were cut into pieces of 3 to 4 mm length. 24 of such pieces were put into the device shown in the figure, where, establishing a contact with 1% hydrogen peroxide solution, we determined the volume of O_2 released during 1 minute. Activity was displayed mainly on the wound surface of the leaf pieces, on account of enzyme activity asserting itself there. Approximately, the total wound surface of a sample amounted to 50 sq. mm.

The data were compiled in spring 1962; the data presented are from a measuring performed on May 19th, on a calm, hazy day, with sunshine at noon.

In case of 3 to 5 replicates, the data of samples taken at the same time from similar places varied about the mean value with so small an amplitude that we did not indicate the standard deviation at every record. According to the so called "rule of 3-s" (B r a u n e r – B u k a t s c h 1961), significance was unquestionable, since the fluctuation was far from 1/3 of the mean value. In terms of this rule, a record is considered as significant if its numerical value is more than three times as high as that of the mean error. It was only at the averageing of diurnal fluctuations where we indicated the deviation according to the following well-known formula:

$$s = \pm \sqrt{\frac{\Sigma v^2}{n-1}}$$

The following table shows the diurnal march of the catalase activity of 20 – 25 year old *Pinus nigra* trees, as measured at the apical, medial and basal parts of the needles. The needles were taken at medium height from inside the crown. Divergencies due to position were compensated by the fact that the samples of needles directed towards different points of the compass were placed together into the recipient.

Catalase activity of Pinus nigra needles

Time of experiment (hour, minute) and temperature (C°)	O_2 cu mm/min released from H_2O_2 at the		
	apical	medial	basal
	part of the pine-needle		
1h 00 min. 14 °C	33,3	42,8	78,9
14h 30 min. 23 °C	28,8	35,7	41,3
16h 00 min. 24 °C	27,7	34,8	35,2
19h 20 min. 19 °C	22,1	28,8	31,2
22h 00 min. 18 °C	29,2	38,2	38,2
24h 00 min. 18 °C	30,7	40,8	62,1
Average	28,6 ± 3,7	36,8 ± 4,9	47,8 ± 18,6

A comparison of the figures reveals a distinct diurnal periodicity of activity, as well as a gradient of catalase activity increasing towards the basal part of the pine-needles. As shown numerically by the deviations indicated

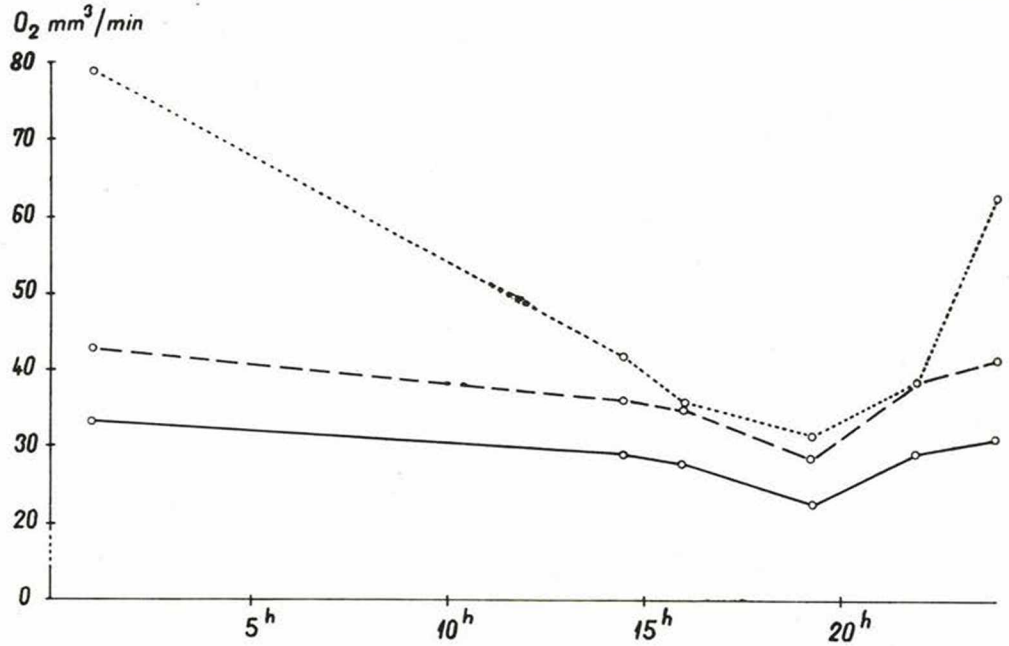

Fig. 2. Diurnal changes of catalase activity in *Pinus nigra* needles. – ..... = apical part; – – – – – = medial part; _____ = basal part

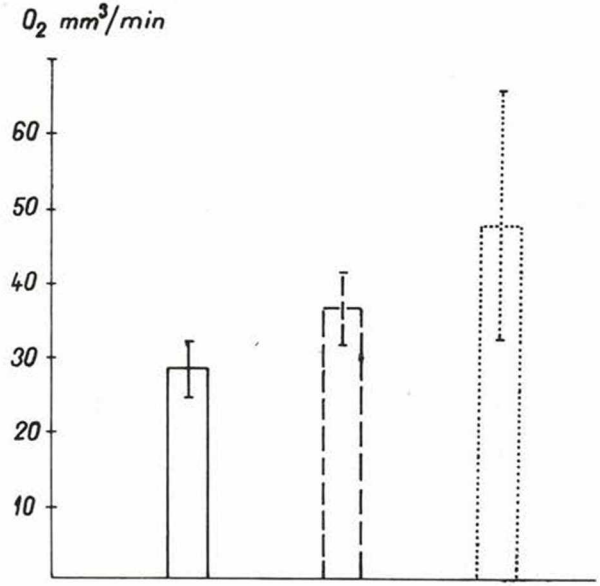

Fig. 3. Gradient of catalase activity in *Pinus nigra* needles. For symbols see Fig. 2. The lign on the columns represents the deviation from the diurnal mean at different sections of the day, with indication of the standard deviation instead of the extreme values

next to the daily mean values, the greatest diurnal fluctuation of activity takes place in the physiologically youngest basal parts of the pine-needle where catalase activity is strongest. All this is shown on the following diagrams (Fig. 2 and 3.).

Discussion

The unequal distribution of the sampling times is objectionable, introducing a factor of uncertainity into the determination of the daily mean value. However, it is unquestionably correct to presume a diurnal rhythm in the catalase activity of the tested pine-needles, because, from one night to the other, catalase activity approaches the initial starting value in each of the three parts of the needle. Discussed at the Vth meeting of the Hungarian Biological Society, the studies on the phenomena of rhythm (Mödlinger 1963, Frenyó 1963, etc.) end with the conclusion that periodical alterations can be detected practically in every vital process. A certain correlation can be presumed to exist between the catalase rhythm observed in the pine-needle and the diurnal rhythm of photosynthesis, but this cannot be declared categorically only if besides the light conditions temperature too has changed. In the case of *Pinus montana*, not mentioned in the experimental part, we have observed a somewhat similar daily trend of catalase activity, although the gradient within the pine-needle is essentially dissimilar to the type described in connection with *Pinus nigra*. The hypothesis of a possible correlation existing between the catalase activity of pine-needles and photosynthesis is supported by the similar reaction of two different types to the diurnal alterations of light, but this has not been accurately investigated so far.

It is also objectionable that, when indicating the deviation from the daily mean on Fig. 3., we are actually indicating the standard deviation instead of showing the lowest diurnal and the highest nocturnal catalase activity. Notwithstanding, we have decided in favour of this solution, because otherwise the characterization of the daily mean value would be uncertain.

In the scope of afforestation programs, phytophysiological and biochemical tests on forest trees are of increased importance. The catalase rhythm and catalase gradient observed in the pine-needle remind us that, in case of diagnostical examinations, sampling must be accomplished with due consideration taken of the daily rhythm and of topographical deviations. Most rigorous implementation of sampling is a requirement that must be met unless entirely false results may be obtained. In some of our tests, unpublished here, we have observed in certain cases a difference of almost 700% in the catalase activity of apical and basal leaf tissues, if the test was affected also by laboratory conditions. Catalase measurements may be considered as correct only if performed in the field and within short time, because – according to our observation – the catalase activity of plant tissues and, most of all, that of leaf tissues is changing very rapidly. The method described in the present paper worked well for field tests.

Summary

By means of our catalase-testing equipment suitable for field measurements, we have demonstrated in the needles of Pinus nigra a gradient of catalase activity, increasing in the average by approximately 67% from the older distal (apical) part to the younger proximal (basal) part. Catalase activity also shows a periodical diurnal fluctuation which appears to depend mainly on light (activity is strongest at night). The amplitude of diurnal fluctuation is the widest in the tissues of the youngest (proximal) part of the pine-needles where metabolism is the most intensive.

On one hand, these alterations may be in some correlation to photosynthesis, thus possibly informing us on metabolic changes due to photosynthesis and on the other hand, they remind us of normal topographical differences of leaf samples taken for the purposes of biochemical tests.

Résumé

À l'aide de notre équipement pour l'examen de la catalase, se prêtant aux mesurages in situ, nous avons démontré dans les aiguilles de *Pinus nigra* un gradient de l'activité de catalase, augmentant dans la moyenne d'environ 67% à partir de la partie distale (apicale) plus âgée jusqu'à la partie basale (proximale) plus jeune. L'activité de catalase montre aussi une fluctuation périodique diurne qui semble dépendre en premier lieu de la lumière (pendant la nuit l'activité est la plus grande). L'amplitude de la fluctuation diurne est la plus large dans les tissus de la partie la plus jeune (proximale) de l'aiguille, où le métabolisme est le plus intense.

D'une part, ces altérations peuvent être dans une correlation quelconque avec la photosynthèse et nous informer, le cas échéant, de changements métaboliques produits de la photosynthèse, et d'autre part, elles attirent notre attention sur les changements topographiques réguliers des échantillons de feuilles, pris pour des analyses biochimiques.

РЕЗЮМЕ

На основе местных измерений при помощи нашего пригодного для этой цели прибора для исследования каталазы в иголках Pinus nigra нами доказан такой градиент каталазной активности, которая исходя из более старой (верхушечной, дистальной) части к более молодой основной (проксимальной) части в среднем увеличивается примерно на 67 проц. В каталазной активности наблюдается даже и ежедневное колебание, которое кажется зависимым в первую очередь от света. (В ночное время активность является наибольшей). Амплитуда ежедневного колебания является наиболее широкой в тканях наиболее молодых (проксимальных) частей еловой иголки, где и обмен веществами кажется наиболее интенсивным.

Эти изменения могут быть, с одной стороны, в какой-нибудь корреляции с фотосинтезом, следовательно, они случайно могут дать информации о таких изменениях в обмене веществами, которые происходят в результате фотосинтеза, но, с другой стороны, они обращают наше внимание на закономерные топографические различия в образцах листьев, взятых для биохимических исследований.

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